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(54) Title: ANTIMICROBIAL AGENTS FOR ORAL HYGIENE PRODUCTS

(57) Abstract

Oral hygiene compositions include an antimicrobial agent selected from cedarwood oil, chloramphenicol, citronella oil, *Glycyrrhiza glabra* extract, juicy fruit basil oil, lemon basil oil, and *Rosmarinus officinalis* oil. Application of these oral hygiene compositions to the oral cavity effectively reduces or prevents the growth of bacteria associated with dental plaque, and with dental caries and/or periodontal diseases such as *Actinomyces viscosus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis*.

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ANTIMICROBIAL AGENTS FOR ORAL HYGIENE PRODUCTS

Field of the Invention

The present invention relates to antimicrobial agents for use in oral hygiene products and methods for using such agents.

Background of the Invention

Periodontal disease and dental caries are of major public health and economic interest worldwide. It is now widely recognized that both of these oral diseases are caused by bacteria which grow in masses on the teeth and in the gingival area. A commonly used descriptive term for these bacterial masses is "dental plaque". In the case of periodontal disease, Schluger et al. (Schluger, Yuodelis, Page & Johnson, *Periodontal Diseases,* second edition, pp. 153-262, Lea & Febiger, 1990) report that dental plaque bacteria, growing in the area where the teeth and gingival tissues meet, cause an inflammation of the gingiva called "gingivitis". This is characterized by swollen, edematous gingiva ("gums") which are reddened and bleed easily. If plaque removal is inadequate, gingivitis may progress to "periodontitis" or periodontal disease in many individuals. Periodontitis generally is a characterized by a chronic inflammation of the tissues around the teeth, which leads to a resorption of supporting bone. Periodontal disease is the leading cause of tooth loss among adults. Dental caries (cavities) are also caused by bacteria, with *Streptococcus mutans* being the principal etiologic agent (McGhee, Michalek & Cassell, *Dental Microbiology*, p. 279, Harper & Row, 1982).

The prevention of dental plaque or the removal thereof has long been the focus of development with the ultimate goal of inhibiting both caries and periodontal diseases. While the formation of dental plaque can be inhibited to a certain extent by

brushing the teeth at frequent intervals, brushing alone is not sufficient to effectively prevent the formation of dental plaque or remove substantially all of the dental plaque that has formed on the teeth.

Since brushing alone is not sufficient to prevent and remove plaque, chemical methods using antibacterials such as chlorhexidine, benzalkonium chloride, and cetylpyridinium chloride have been proposed. In addition, the use of natural products for the treatment of teeth and gums is old in the art, having been practiced and documented since the mid-1880s. Since then, numerous patents have disclosed compositions of oral products containing natural product extracts. There are numerous natural essential oils available. Many of these oils are described in KIRK OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY, 4th ed., vol. 17, pp. 603-674, John Wiley & Sons, Inc. Morton Pader, in "Oral Hygiene Products and Practice," *Cosmetic Science and Technology Series*, vol. 6, at pp. 356-373, Marcel Dekker, Inc., describes sanguinaria extract as an anti-plaque agent with antimicrobial properties. Pader also describes that volatile oils such as eucalyptol, menthol, thymol, methylsalicylate have varying degrees of antimicrobial activity, and antiplaque activity has been reported under appropriate test conditions. Pader describes that cinnamon oil is a very weak antiseptic, and that eucalyptus oil and eucalyptol are antiseptic. Pader notes that some essential oils are used in other products primarily for flavor. Among these are cinnamon, cassia, clove, thyme, peppermint, anise and anethol. Pader also describes that these essential oils have detectable antimicrobial activity.

For example, it is known that cocamidopropyl betaine, hinokitiol, and berberine and the essential oils, citral, geraniol, and juniper berries oil individually exhibit antimicrobial properties against certain bacteria.

U.S. Patent No. 3,940,476 describes a method for inhibiting the formation of dental plaque, which comprises topically applying to the teeth as an active ingredient an amount of either one or a combination of (a) allyl isothiocyanate, (b) uranine, (c) obtusastyrene, (d) citral, (e) citronellol, (f) nerol, or (g) geraniol.

U.S. Patent No. 4,913,895 describes an oral composition including a linear polyphosphate or a cyclic polyphosphate and menthol, anethol, or mixtures thereof in an aqueous medium. The composition is reported to have antibacterial effects and prevent the development of calculus and periodontal diseases.

U.S. Patent No. 4,966,754 describes that certain essential oils and combinations thereof possess antimicrobial properties against *Aspergillus niger*,

Candida albicans, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and therefore are suitable as preservatives in cosmetic compositions. A blend of 14 essential oils is described as providing desirable antimicrobial properties against the noted microorganisms.

U.S. Patent No. 4,999,184 describes oil compositions containing certain pyrophosphate salts which are reported to provide an anticalculus benefit.

U.S. Patent No. 5,316,760 describes a mouth care product that contains a combination of *Urtica dioica* extract and an extract of *Juniperus communis*. The combination of these extracts is described as leading to a synergistic reduction of both dental plaque and bleeding or inflammation of the gingiva. *Achillaea millefolium* extract is also described as being a suitable additive to the combination of the *Urtica dioica* and *Juniperus communis* extracts.

U.S. Patent No. 5,472,684 describes a composition including thymol and eugenol, and optionally a sesquiterpene alcohol, such as farnesol, that reportedly has antiplaque and antigingivitis effects. Australian tea tree oil, sage oil, and eucalyptol are described as enhancing the antiplaque and antigingivitis activity of mouth rinses formulated from the disclosed compositions.

One property that characterizes the effectiveness of an antimicrobial agent as an antiplaque and anticalculus agent is the minimum inhibitory concentration, or MIC, of the agent. The MIC is the minimum concentration in micrograms per milliliter of an antimicrobial agent at which no bacterial growth are observed. At concentrations below the MIC, an antimicrobial agent is ineffective at killing or inhibiting the growth and reproduction of bacteria. At concentrations above the MIC, an antimicrobial agent is effective at killing or inhibiting the growth and reproduction of bacteria.

Typically, antimicrobial agents are introduced into the oral cavity at an initial concentration. Almost immediately, the initial concentration begins to decrease because of the dynamics of the oral cavity. Eventually, the concentration of the antimicrobial agent within the oral cavity will fall below the MIC. Thus, it has been a goal of those working to develop antiplaque and anticalculus formulations to use antimicrobial agents that have low MICs.

Chlorhexidine has a MIC of about one μg/ml and is the standard against which other antimicrobial agents are measured. While chlorhexidine has a desirable MIC, it also exhibits undesirable taste and has the undesirable side effect of staining teeth.

Summary of the Invention

In one aspect, the present invention is an oral hygiene composition that includes an antimicrobial agent selected from cedarwood oil, chloramphenicol, citronella oil, *Glycyrrhiza glabra* extract, juicy fruit basil oil, lemon basil oil, and *Rosmarinus officinalis* oil. According to the present invention, oral hygiene compositions including these antimicrobial agents are effective at inhibiting and preventing the growth of bacteria present in oral cavities such as *Actinomyces viscosus, Fusobacterium nucleatum, Porphyromonas gingivalis, Streptococcus mutans,* and *Streptococcus sanguis.* Oral hygiene compositions formed in accordance with the present invention are characterized in that the antimicrobial agent is present in an amount sufficient to retard the growth of bacteria or kill bacteria.

The antimicrobial agents can be combined with components typically found in oral hygiene products such as dentifrices. These components include abrasives, humectants, binders, and surfactants. Other dentifrice components include flavoring agents and thickening agents.

In accordance with the present invention, the oral hygiene compositions and products of the present invention can be used in the method of practicing oral hygiene that includes the step of contacting an oral cavity with an antimicrobial agent selected from cedarwood oil, chloramphenicol, citronella oil, *Glycyrrhiza glabra* extract, juicy fruit basil oil, lemon basil oil, lemon oil, or *Rosmarinus officinalis* oil.

Detailed Description of the Preferred Embodiment

As used herein, the following terms have the following meanings.

"Cedarwood oil" refers to volatile whole oil extracts from the heartwood of *Juniperus virginiana* or *Juniperis ashei.* Constituents of the whole oil extract include thujopsene, cedrol, alpha-copaene, alpha-cedrene, beta-cedrene and widdrol. The CAS number for cedarwood oil is 8000-27-9.

"Chloramphenicol" refers to 2,2-dichloro-N-[2-hydroxyl-1-(hydroxymethyl)-2-(4-nitrophenyl) ethyl] acetamide. Chloramphenicol is derived from *Streptomyces venezuelae* or by organic synthesis. The CAS number for chloramphenicol is 56-75-7.

"Citronella oil" refers to a commercially available oil produced by steam distillation of either *Cymbopogon nardus* or *Cymbopogon winterianus.* The CAS number for citronella oil is 8000-29-1.

"*Glycyrrhiza glabra* extract", also known as licorice root extract, refers to the crude powder extract from *Glycyrrhiza glabra* L. Several varieties including *G. typica* and *G. glandulifera* exist. *Glycyrrhiza glabra* extract contains glycyrrhizic acid and glycyrrhetinic acid. The whole extract is available from commercial sources or may be collected by solvent extraction, such as ethanol extraction described below.

"Juicy fruit basil oil" refers to the whole extract of a selected variety of basil with a juicy fruit component. Juicy fruit basil is a cultivar of *Ocimum bascilicum L.*

"Lemon basil oil" refers to the volatile whole oil extract from a selected variety of basil with a citral component. Lemon basil is a cultivar of *Ocimum bascilicum L.* with a high content of citral.

"Lemon oil" refers to the volatile whole oil extract from the fresh peel of *Citrus limon.* Lemon oil is also known as oil of lemon or citrus limon oil. The CAS number for lemon oil is 8008-56-8.

"*Rosmarinus officinalis* oil" refers to the whole oil extract from the flowering tops of *Rosmarinus officinalis*. *Rosmarinus officinalis* oil is also known as the extract of Rosemary, or the extract of *Rosmarinus officinalis* oil. The CAS number for *Rosmarinus officinalis* oil is 84604-14-8.

All of the foregoing are available from commercial sources.

"Minimal inhibitory concentration or MIC" refers to the minimum concentration in micrograms per milliliter of an antimicrobial agent at which no bacterial growth are observed. At concentrations below the MIC, the antimicrobial agent is ineffective at killing or inhibiting the growth and reproduction of bacteria. At concentrations above the MIC, the antimicrobial agent is effective at killing or inhibiting the growth and reproduction of bacteria.

An oral hygiene composition formed in accordance with the present invention includes an antimicrobial agent selected from cedarwood oil, chloramphenicol, citronella oil, *Glycyrrhiza glabra* extract, juicy fruit basil oil, lemon basil oil, and *Rosmarinus officinalis* oil. These oral hygiene compositions can be incorporated into oral hygiene products formulated in accordance with the present invention, such as dentifrices, mouth washes, and mouth rinses.

Preferred oral hygiene compositions include an antimicrobial agent selected from cedarwood oil, chloramphenicol, and *Glycyrrhiza glabra* extract. Particularly preferred for incorporation into an oral hygiene composition formulated in accordance with the present invention are antimicrobial agents selected from

cedarwood oil and *Glycyrrhiza glabra* extract. The preferred antimicrobial agents are selected because these agents are surprisingly effective at retarding the growth of and/or preventing the growth of representative gram-positive and gram-negative oral pathogenic bacteria such as *Actinomyces viscosus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis*. The examples that follow illustrate the effectiveness that the antimicrobial agents of the present invention have against these particular bacteria. Preferably, the antimicrobial agent is effective against more than one of the bacteria noted above, and preferably all of the bacteria noted above.

The particular amount of antimicrobial agent present in compositions formed in accordance with the present invention is not limited to any particular value, provided that the amount present is effective at retarding the growth of bacteria and/or preventing the growth of bacteria, i.e., an amount that is greater than the MIC of the antimicrobial agent with respect to the particular bacteria.

As illustrated in the examples that follow, the antimicrobial agents of the present invention exhibit MICs that range from about 3.1 to about 156 against the representative oral pathogens *Actinomyces viscosus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis*.

Dentifrices, or toothpastes, are generally a thickened slurry of an abrasive polishing material in an aqueous humectant system. Typically, dentifrices include an abrasive to remove stained pellicle, humectant(s) to provide a vehicle for the flavor, abrasive, thickening agent(s) to structure and stabilize the dentifrice, surfactant mainly to supply foam during use, fluoride to prevent dental caries, and flavor to make the product taste pleasant.

Numerous abrasives are available for use in dentifrices, examples include silica xerogel, silica precipitates, dicalcium phosphate, dicalcium phosphate dihydrate, alumina trihydrate, calcium pyrophosphate, calcium carbonate, and insoluble sodium metaphosphate.

Examples of suitable humectants include sorbitols, glycerin, and polyethylene glycols.

Silica aerogels, pyrogenic silica, silica precipitates, carboxymethylcellulose, carboxyvinyl polymers, xanthan gum, and carrageenan are examples of materials that are suitable as thickeners.

Exemplary surfactants include sodium lauryl sulfate and dodecylbenzene sulfonate.

Numerous flavoring agents are commercially available with those providing mint or other refreshing flavors such as cinnamon being commonly used.

Oral rinses or mouth washes are generally, aqueous, pourable emulsions of flavors into which, in most instances, an antimicrobial has been incorporated. Typical components of an oral rinse include flavoring agent to make the product pleasant to use and to emphasize therapeutic or freshness qualities, surfactant(s) to maintain flavor in stable dispersion, humectant(s) to improve mouth feel, thickening agent, and an active agent. Often times, a surfactant is used to impart light foaming properties to the oral rinse.

Dentifrices and oral rinses incorporating the antimicrobial agents for inhibiting the growth of bacteria in the oral cavity in accordance with the present invention are formulated in a conventional manner with the antimicrobial agents being present in an amount ranging from about 0.001 wt. % to about 5.0 wt. % based on total weight of dentifrice. Preferably the antimicrobial agents are present in an amount ranging from about 0.01 wt. % to about 2.5 wt. %. The specific components used in the dentifrices and oral rinses incorporating the antimicrobial agents of the present invention are not limited to those set forth above; however, the components selected preferably do not have an antagonistic effect on the antimicrobial properties of the selected antimicrobial agent.

The following examples illustrate the effectiveness of oral hygiene compositions of the present invention against bacteria present in the oral cavity, the MIC for the antimicrobial agents in the compositions against such bacteria, dentifrice formulations including antimicrobial compositions of the present invention, consumer preference for such dentifrice formulations, and *in vivo* effectiveness data for such dentifrice formulations.

Example 1

Determination of Minimum Inhibitory Concentration of Antimicrobial Agents

The following example illustrates how antimicrobial agents useful in accordance with the present invention retard or prevent the growth of dental plaque bacteria present in the oral cavity. In addition, the example illustrates the lowest concentration of various antimicrobial agents that will inhibit visible *in vitro* growth of a particular bacteria.

The assay used a microtiter plate to dilute the antimicrobial agent to varying concentrations in order to determine the MIC.

Table 1 below provides a listing of antimicrobial agents used in this example and abbreviations therefor.

Table 1

Antimicrobial Agents and Abbreviations Therefor

cedarwood oil	(RC1)
chloramphenicol	(CR1)
citronella oil	(CTR1)
Glycyrrhiza glabra extract	(GLY)
juicy fruit basil oil	(JFB1)
lemon basil oil	(LMB1)
Rosmarinus officinalis oil	(ROF1)

A bacteria culture was incubated overnight at 37°C. Prior to dilution of the antimicrobial agents as described below, the bacterial culture was spun down at 2000 rpm into a pellet and resuspended in a solution of buffered phosphate. The innoculum was normalized with a spectraphotometer to an optical density at 550 nanometers of between 0.18-0.22, equivalent to 5.0×10^7 colony-forming units (CFU per milliliter). The innoculum was set aside until the completion of the antimicrobial agent dilution.

A sterile polystyrene 96-well plate was used to dilute the antimicrobial agents. Using aseptic technique, 100 microliters of distilled water was placed in each test well. In the first well in each column, 100 microliters of antimicrobial agent was added. Stock solutions of antimicrobial agents were prepared with methanol as a solvent to bring the agents into solution. This resulted in a one-half dilution of the stock solution. 100 microliters from these wells was then transferred to the next well in the column, and so on, down each column. Each transfer accomplished a one-half dilution of the concentration in the preceding well. After the dilution of the antimicrobial agent was completed, 80 microliters of growth media specific for the bacteria under study was added to each well. The specific growth media for a given bacteria are set forth in Table 2.

Next, 20 microliters of innoculum was added to each well. This resulted in the first well of each column having a final dilution of one quarter of the stock solution. The remaining wells were a one-half dilution of the preceding well for each transfer.

The 96-well plate was incubated under conditions that varied depending on the particular microorganism. The incubation conditions are set forth in Table 2.

The aerobic bacteria were incubated under normal room conditions and the anaerobic bacteria were incubated under an atmosphere of 10% hydrogen gas, 5% carbon dioxide gas and the balance nitrogen gas. Following 48 hours of incubation, the incubated plate was read for microbial growth with a spectraphotometer by optical density (OD). The well containing the lowest dilution achievable with a spectraphotometer reading below 0.05 OD (i.e., no detectable microbial growth) was considered representative for the antimicrobial agent. The MIC for the agent was determined by accounting for the starting stock solution concentration and the resulting dilutions in the 96-well plate.

The specific bacteria inoculated into the 96-well plate are set forth in Table 2 below, along with the growth media and incubation conditions for that microorganism.

Table 2

Microorganisms/Growth Media/Incubation Conditions

Microorganism	ATCC No.	Growth Media	Incubation Conditions
Campylobacter rectus	33238	CR media[4]	48 hrs/37°C/anaerobic
Actinomyces viscosus (AV)	19246	TSB[1]	48 hrs/37°C/aerobic
Fusobacterium nucleatum (FN)	10933	FN media[3]	48 hrs/37°C/anaerobic
Porphyromonas gingivalis (PG)	33277	PG media[2]	48 hrs/37°C/anaerobic
Streptococcus mutans (SM)	25175	TSB[1]	48 hrs/37°C/aerobic
Streptococcus sanguis (SS)	49295	TSB[1]	48 hrs/37°C/aerobic

[1] Tryptic Soy Broth 3.0% wt. to vol., yeast extract 0.1%, and 999 milliliters distilled water.

[2] Tryptic Soy Broth 3.0% wt. to vol., yeast extract 0.5%, L-cystein 0.05%, Hemin 0.0005%, Menadione 0.00002%, and 990 milliliters distilled water.

[3] Tryptic Soy Broth 3.0% wt. to vol., yeast extract 0.5%, Peptone 1.0%, L-cystein extract, Hemin 0.0005%, Menadione 0.00002%, and 990 milliliters distilled water.

[4] Brain Heart Infusion Broth 0.74% wt. to vol., yeast extract 0.01%, sodium formate 0.2%, sodium fumerate 0.03%, hemin 0.005% and 990 millileters distilled water.

In the following tables, the antimicrobial agents are identified with respect to the abbreviations set forth in Table 1. In addition, the five microorganisms set forth in Table 2 are referred to by the abbreviations set forth in Table 2.

Table 3

MIC (μg/ml) for Antimicrobial Agents for Indicated Bacteria

Agent	AV	CR	FN	PG	SM	SS
RC1	31.3	31.3	31.3	7.8	31.3	15.6
CR1	3.1	-	3.1	6.3	3.1	3.1
CTR1	62.5	125	31.3	31.3	62.5	31.3
GLY	15.6	15.6	15.6	7.8	15.6	7.8
JFB1	156.3	156.3	156.3	62.5	156.3	156.3
LMO1	312.5	125	62.5	31.3	125	125
LCC1	312.5	125	156.3	78.1	156.3	156.3
ROF1	125	125	62.5	62.5	125	125

This example illustrates the minimum inhibitory concentration of the noted antimicrobial agents and the ability of the antimicrobial agents to inhibit growth of the specific bacteria.

Example 2

Dentifrice Formulations

This example describes dentifrice formulations comprising antimicrobial agents of the present invention. Formulation 18-88 does not include antimicrobial agents in accordance with the present invention.

Table 4

Formulation 5-82: 0.1% Cedarwood Oil (W/W)

Component	Weight Percent
Sorbitol 70%	37.00
Poloxamer 407 (PLURONIC® F127)	9.50
Deionized Water	24.50
Carbomer 940 (CARBOPOL® 940)	0.30
Sodium Hydroxide	0.20

Component	Weight Percent
Xanthan Gum	0.40
Glycerin	4.75
Sodium Fluoride	0.25
Sodium Saccharin	0.30
SYLODENT® 750 (silica)	9.50
SYLODENT® 15 (silica)	9.50
Cedarwood oil	0.50
Flavor	0.90
FD&C Blue #1 1% solution	0.10
Titanium Dioxide	0.90
Sodium Lauryl Sulfate	1.40

Table 5

Formulation 5-81: 0.1% Cedarwood Oil (W/W)

Component	Weight Percent
Sorbitol 70%	58.63
Sodium Carboxymethylcellulose	0.35
Deionized Water	3.00
Polyethylene Glycol 600	5.00
Glycerin	10.00
Sodium Fluoride	0.22
Sodium Saccharin	0.30
Sodium Benzoate	0.50
SYLODENT® 700 (silica)	15.00
AEROSIL® 200 (silica)	3.00

Component	Weight Percent
Flavor	0.80
Ethanol	1.50
Cedarwood	0.10
FD&C Blue #1 1% solution	0.10
Sodium Lauryl Sulfate	1.50

Table 6

Formulation 5-108: 0.4% Cedarwood Oil (W/W)

Component	Weight Percent
Sorbitol 70%	50.00
Deionized water	24.63
Carbomer 940 (CARBOPOL® 940)	0.30
Sodium hydroxide	0.20
Xanthan gum	0.500
Sodium fluoride	0.22
Sodium saccharin	0.55
SYLODENT® 750 (silica)	10.00
SYLODENT® 15 (silica)	10.00
Cedarwood oil	0.40
Flavoring agents	0.80
Titanium dioxide	1.00
Sodium lauryl sulfate	1.40

Table 7

Formulation 18-87: 0.5% *Glycyrrhiza glabra* Extract (W/W)

Component	Weight Percent
Sorbitol 70%	50.00
Deionized water	24.53
Carbomer 940 (CARBOPOL® 940)	0.30
Sodium hydroxide	0.20
Xanthan gum	0.50
Sodium fluoride	0.22
Sodium saccharin	0.55
SYLODENT® 750 (silica)	10.00
SYLODENT® 15 (silica)	10.00
Glycyrrhiza glabra extract	0.50
Flavoring agents	0.80
Titanium dioxide	1.00
Sodium lauryl sulfate	1.40

Table 8

Formulation 18-90: 1% Cedarwood Oil (W/W)

Component	Weight Percent
Sorbitol 70%	50.00
Deionized water	24.03
Carbomer 940 (CARBOPOL® 940)	0.30
Sodium hydroxide	0.20
Xanthan gum	0.50
Sodium fluoride	0.22
Sodium saccharin	0.55
SYLODENT® 750 (silica)	10.00
SYLODENT® 15 (silica)	10.00

Component	Weight Percent
Cedarwood oil	1.00
Flavoring agents	0.80
Titanium dioxide	1.00
Sodium lauryl sulfate	1.40

Table 9

Formulation 18-25: 0.1% Hinokitiol

Component	Weight Percent
Deionized Water	92.02
Carbomer 940 (CARBOPOL® 940)	1.00
Sodium Hydroxide	0.75
Sodium Saccharin	0.50
Sodium Fluoride	0.23
Flavor	0.80
Hinokitiol	0.10
Ethanol	3.50
Sodium Lauryl Sulfate	1.10

Table 10
Formulation 18-88: Control

Component	Weight Percent
Sorbitol 70%	37.70
Poloxamer 407 (PLURONIC® F127)	9.50
Deionized Water	25.50
Carbomer 940 (CARBOPOL® 940)	0.30
Sodium Hydroxide	0.20
Xanthan Gum	0.40
Glycerin	4.75
Sodium Fluoride	0.25
Sodium Saccharin	0.40
SYLODENT® 750 (silica)	9.50
SYLODENT® 15 (silica)	9.50
Titanium Dioxide	1.00
Sodium Lauryl Sulfate	1.00

"Carbomer" refers to a polymer composed of acrylic acid crosslinked with allyl sucrose available as CARBOPOL® 940. CARBOPOL® 940 is commercially available from B.F. Goodrich. SYLODENT® 750 and 700 are silica gels (silicon dioxide), and SYLODENT® 15 is a silica gel (silicon dioxide). SYLODENT® is available commercially from W.R. Grace & Co. Conn. Davison Chemical Division.

The formulations described above are formed in a vacuum mixer by adding deionized water and dispersing the Carbomer while pulling a vacuum. When the Carbomer was well dispersed, the sodium hydroxide was added. The 70% sorbitol and Poloxamer were heated and mixed in another vessel. The Poloxamer mixture was then added to the vacuum mixer and blended with the Carbomer mixture. Xanthan gum was mixed with glycerin and then added to the solution in the vacuum mixer and incorporated therein. The salts were then added to the vacuum mixer

followed by the silicas. These components were slowly mixed in. Next, the active agents, flavoring agents, sodium lauryl sulfate and coloring agents were added followed by mixing until well incorporated.

This example illustrates several dentifrice formulations incorporating antimicrobial agents of the present invention.

Example 3

Customer Acceptance

This example illustrates customer acceptance of dentifrice formulations incorporating an antimicrobial agent in accordance with the present invention.

The formulations set forth above in Tables 4-9 were evaluated for customer acceptance. For comparison purposes, several commercially available dentifrices were also evaluated. These included Viadent Gel (Formulation 5-35), available from Viadent, Inc.; Crest Regular Blue Paste (Formulation 5-97), available from Proctor & Gamble Company; and Listerine Teal Gel (Formulation 5-99), available from Warner Wellcome.

The preference study was carried out with a group of approximately 20 people. One tube of dentifrice formulation was given to each person to use over the course of a week. At the end of the week, participants filled out a questionnaire, the results of which are summarized in Table 11 below. The scoring range was 1-10, with 1 being "poor" and 10 being "good." The scores were tallied and averaged.

Table 11

Consumer Evaluation

Formulation	5-82	5-81	5-108	5-35	5-97	5-99
Color	6.61	8.00	6.75	2.72	6.64	7.25
Appearance	6.42	7.90	6.90	3.16	6.62	6.75
Taste	5.80	6.20	7.15	3.72	6.27	4.00
After Taste	6.80	5.85	6.05	3.38	6.09	3.85
Aroma	7.19	6.79	7.05	4.33	6.32	4.55
Cleaning	7.38	5.95	7.00	3.94	6.41	6.10
Foam	6.42	5.10	7.55	3.66	6.05	5.85

Formulation	5-82	5-81	5-108	5-35	5-97	5-99
Consistency	7.23	5.95	7.70	4.00	6.14	5.75
Dry Mouth	8.14	6.90	7.10	3.89	7.91	5.70
Next Morning Feel	7.00	6.16	7.05	3.39	5.50	5.15
Overall Mouth Feel	7.33	6.16	7.05	3.11	6.09	4.45
Overall Satisfaction	6.90	6.10	6.90	3.05	5.95	4.20

This example illustrates how the overall consumer satisfaction for the formulations with active agents in accordance with the present invention is greater than the overall consumer satisfaction for certain commercially available products.

Example 4

In Vivo Gingival Margin Bacterial Reduction

This example illustrates how dentifrice formulations including compositions of the present invention reduce bacteria levels in the gingival margin.

In this example, the subject's brushing habits remained constant. On the day of sampling, subjects had not brushed their teeth for 16-18 hours (overnight) prior to testing. The level of bacteria was enumerated by recovering *in vivo* samples and counting the number of bacterial colonies formed on agar after 48 hours.

Subjects were 18-65 years old, had a minimum of 20 teeth, and had normal salivary flow. Subjects were rejected if they had systemic diseases that affect the oral tissues, such as diabetes, blood cell abnormalities, Down's syndrome, or known HIV-infection; had glossitis, moderate to severe gingivitis, periodontitis or other oral infections, or were taking a systemic antibiotic or had done so in the two weeks preceding the initiation of the test.

Each of the formulations was assayed for antimicrobial efficacy on approximately 20 subjects. The subjects were requested to have overnight plaque the morning they were sampled for a "single brushing effect" of a formulation.

At baseline, a gingival margin plaque sample was taken with a sterile cotton swab and placed in 1 milliliter of sterile phosphate buffered saline (PBS). Swabs were gently rubbed along the gingival margin of the mandibular buccal surface between the incisors. Subjects then brushed immediately after the baseline sample with a dentifrice formulation using an electric toothbrush for two minutes. At

intervals of one and two hours after completion of the brushing, gingival plaque samples were recovered with swabs as described above.

The clinical design was a multi-period crossover study. By multi-period crossover study is meant an experiment in which subjects are administered first one treatment, then "crossed over" to receive a second, then third and finally a fourth treatment. The experimental design enables each subject to serve as his or her own control. A "wash-out" period of 2 days was used between testing different antibacterial formulations.

The gingival samples were placed in test tubes and remained at room temperature until processed within one hour. The test tube samples were serially diluted in sterile PBS with a Spiral Plater (Spiral Systems, Cincinnati, Ohio) and plated on blood agar supplemented with 5% sheep blood (BBL, Becton Dickinson, Cockeysville, Maryland). The plated samples were then incubated aerobically at 37°C for 48 hours. Bacteria were counted for total aerobic colony forming units.

The bacterial counts were transformed into $\log_{10}$ units. Mean and standard deviations for each test condition were generated. Data was paired by subject for relevant comparisons and analyzed by the nonparametric Wilcoxon sign-rank test for significance level. Hypothesis testing relied on statistics utilizing the multiperiod crossover design of the clinical study.

The data are presented below in Table 12. Formulations 18-25, 18-87, and 18-90 included components set forth in Tables 9, 7 and 8, respectively. Formulation 18-88 included the components set forth in Table 10 (no antibacterial agent) and was used as a control for comparison purposes against formulations 18-25, 18-87 and 18-90.

Table 12

Effect of Antimicrobial Agent Containing Dentifrice Formulations on *In Vivo* Bacterial Levels

Formulation	Active Ingredient	% Conc. wt./wt.	($1x10^6$ CFU/ml) Time:		% Bacterial Reduction vs. Control	Significance Level	Number of Subjects in Trial
			Baseline	2 hrs.			
18-25	Hinokitiol	0.1	33.11	4.47	49.8%	**	17
18-87	*Glycyrrhiza glabra* ext.	0.5	39.81	2.14	76.0%	***	17
18-90	Cedarwood	1.0	31.62	3.16	64.5%	***	17
18-88	Control	--	33.88	8.91	NA	NA	17

Statistical Results: Significance testing was against Control (formulation 18-88) at 2 hours. Wilcoxon sign-rank matched pairs with $\log_{10}$ transformed data.

***$p<0.01$, **$p<0.05$, *$p<0.1$

NA = Not Applicable

This example illustrates how the dentifrice formulations including antimicrobial agents of the present invention reduce gingival *in vivo* bacterial levels.

Example 5

Ethanol Extraction of *Glycyrrhiza glabra*

25 grams of powdered plant material from *Glycyrrhiza glabra* was combined with 250 grams of a 95:5 ethanol/water mixture. The mixture was stirred overnight at room temperature. Solids were removed from the stirred mixture with a No. 4 Whatman filter in a Buchner funnel. Further removal of solids was achieved with a No. 5 Whatman filter in a Buchner funnel. Additional solids were removed with a Whatman 1 micrometer filter in a Buchner funnel. A vacuum filtration apparatus and a 0.2 micrometer filter was employed to clean the solution a final time. The clean solution was then concentrated down to a solid using a rotovaporizer.

Approximately, 2.5 grams of a rust colored solid was collected as the crude extract of *Glycyrrhiza glabra*.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An oral hygiene composition comprising:

an antimicrobial agent selected from the group consisting of cedarwood oil, chloramphenicol, citronella oil, *Glycyrrhiza glabra* extract, juicy fruit basil oil, lemon basil oil, and *Rosmarinus officinalis* oil.

2. The oral hygiene composition of Claim 1, wherein the antimicrobial agent is selected from the group consisting of cedarwood oil, chloramphenicol, and *Glycyrrhiza glabra.*

3. The oral hygiene composition of Claim 2, wherein the antimicrobial agent is selected from the group consisting of cedarwood oil and *Glycyrrhiza glabra.*

4. The oral hygiene product of Claim 1, wherein the antimicrobial agent is present in an amount effective to retard growth of oral pathogenic bacteria.

5. The oral hygiene composition of Claim 4, wherein the oral pathogenic bacteria are selected from the group consisting of *Actinomyces viscosus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis.*

6. The oral hygiene composition of Claim 1, wherein the antimicrobial agent is present in an amount effective to kill bacteria.

7. The oral hygiene composition of Claim 6, wherein the bacteria are selected from the group consisting of *Actinomyces viscosus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis.*

8. The oral hygiene composition of Claim 1, wherein the antimicrobial agent is present in an amount effective to inhibit the formation of dental plaque.

9. The oral hygiene composition of Claim 1, wherein the antimicrobial agent is present in an amount greater than the minimum inhibitory concentration of the agent.

10. A dentifrice comprising:

an antimicrobial agent selected from group consisting of cedarwood oil, chloramphenicol, citronella oil, *Glycyrrhiza glabra*, juicy fruit basil oil, lemon basil oil, lemon oil, and *Rosmarinus officinalis* oil;

an abrasive;

a humectant;

a binder; and

a surfactant.

11. The dentifrice of Claim 10, wherein the antimicrobial agent is selected from the group consisting of cedarwood oil, chloramphenicol, and *Glycyrrhiza glabra.*

12. The dentifrice of Claim 11, wherein the antimicrobial agent is selected from the group consisting of cedarwood oil and *Glycyrrhiza glabra.*

13. The dentifrice of Claim 10, wherein the antimicrobial agent is present in an amount effective to retard growth of oral pathogenic bacteria.

14. The dentifrice of Claim 13, wherein the bacteria are selected from that group consisting of *Actinomyces viscosus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis.*

15. The dentifrice of Claim 10, wherein the antimicrobial agent is present in an amount effective to kill oral pathogenic bacteria.

16. The dentifrice of Claim 15, wherein the oral pathogenic bacteria are selected from the group consisting of *Actinomyces viscosus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis.*

17. The dentifrice of Claim 10, wherein the antimicrobial agent is present in an amount effective to inhibit the formation of dental plaque.

18. The oral hygiene product of Claim 10, wherein the antimicrobial agent is present in an amount greater than the minimum inhibitory concentration of the agent.

19. A method for practicing oral hygiene comprising the step:

contacting an oral cavity with an antimicrobial agent selected from the group consisting of cedarwood oil, chloramphenicol, citronella oil, *Glycyrrhiza glabra*, juicy fruit basil oil, lemon basil oil, lemon oil, and *Rosmarinus officinalis* oil.

20. The method of Claim 19, wherein the antimicrobial agent is selected from the group consisting of cedarwood oil, chloramphenicol, and *Glycyrrhiza glabra*.

21. The method of Claim 18, wherein the antimicrobial agent is selected from the group consisting of cedarwood oil and *Glycyrrhiza glabra*.

22. The method of Claim 19, wherein the contacting step further comprises contacting the oral cavity with an amount of the antimicrobial agent effective to retard growth of oral pathogenic bacteria.

23. The method of Claim 22, wherein the oral pathogenic bacteria selected from the group consisting of *Actinomyces viscosus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis*.

24. The method of Claim 19, wherein the contacting step further comprises contacting the oral cavity with an amount of the antimicrobial agent effective to kill oral pathogenic bacteria.

25. The method of Claim 24, wherein the oral pathogenic bacteria is selected from the group consisting of *Actinomyces viscosus*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Streptococcus sanguis*.

26. The method of Claim 19, wherein the contacting step further comprises contacting the oral cavity with an amount of the antimicrobial agent effective to inhibit the formation of dental plaque.

27. The method of Claim 19, wherein the contacting step further comprises contacting the oral cavity with an amount of the antimicrobial agent greater than the minimum inhibitory concentration of the antimicrobial agent.

INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 115, no. 20, 18 November 1991 Columbus, Ohio, US; abstract no. 214829, XP002073809 & JP 03 199 314 A (MARUZEN CHEMICAL CO.) 9 May 1991 see abstract ---	1-10, 19-26
X	DATABASE WPI Week 9605 Derwent Publications Ltd., London, GB; AN 96-045292 XP002073813 & JP 07 309 733 A (KANEBO LTD) , 28 November 1995 see abstract --- -/--	1-9, 19-26

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 015, no. 297 (C-0854), 29 July 1991 & JP 03 109314 A (MARUZEN KASEI CO LTD), 9 May 1991 see abstract	1-4, 8-10, 19-22,24
X	PATENT ABSTRACTS OF JAPAN vol. 012, no. 482 (C-553), 15 December 1988 & JP 63 198616 A (YASUTAKE HICHI), 17 August 1988 see abstract	1-4, 8-10, 19-22,26
X	PATENT ABSTRACTS OF JAPAN vol. 007, no. 143 (C-172), 22 June 1983 & JP 58 057320 A (TSURUI YAKUHIN KOGYO KK), 5 April 1983 see abstract	1-3,8, 19-21,26
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X	PATENT ABSTRACTS OF JAPAN vol. 096, no. 004, 30 April 1996 & JP 07 316064 A (MORISHITA JINTAN KK), 5 December 1995 see abstract	1,8,10, 19,22
X	DATABASE WPI Week 8446 Derwent Publications Ltd., London, GB; AN 84-285014 XP002073812 & JP 59 175 410 A (KANEBO SHOKUHIN KK) , 4 October 1984 see abstract	1,4,19, 22
A	CHEMICAL ABSTRACTS, vol. 122, no. 20, 15 May 1995 Columbus, Ohio, US; abstract no. 248294, XP002073810 & JP 07 025 764 A (POKKA CORP.) 27 January 1995 see abstract	1,4,5,7, 19,22

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